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Effects of Endogenous Ammonia on Neutralization of Inhaled Sulfuric Acid Aerosols

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Effects of Endogenous Ammonia on Neutralization of Inhaled Sulfuric Acid Aerosols

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EFFECTS OF ENDOGENOUS AMMONIA
ON NEUTRALIZATION OF INHALED SULFURIC ACID AEROSOLS

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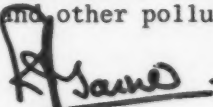
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FOREWORD

The U. S. Environmental Protection Agency was created in response to increasing public concern about the dangers of pollution to the health and welfare of the American people and their environment. The complexities of environmental problems require an integrated program of research and development using input from a number of disciplines.

The Health Effects Research Laboratory was established to provide sound health effects data in support of the regulatory activities of the EPA. An important segment of this data bank includes information delineating the acute effects of inhaled sulfuric acid upon lung function.

This report presents the results of a study designed to determine the effects of increased exogenous and endogenous ammonia upon neutralization of inhaled sulfuric acid in the lungs of dogs. An understanding of the neutralization process and its relationship to lung function is important in determining the potential health effects of sulfuric acid emitted from automobile exhaust and other pollutant sources.



R. John Garner
Director

Health Effects Research Laboratory

ABSTRACT

Nine male beagles were exposed by inhalation to 0, 6, and 10.5 mg/m³ sulfuric acid aerosols with normal ammonia, increased blood ammonia, and increased inhaled ammonia (ammonium sulfate exposures) to determine whether the addition of ammonia affected the toxicity of sulfuric acid aerosols. Toxicity was evaluated from measurements of pulmonary function before, during, and after exposure. Exhaled concentrations of ammonia, sulfuric acid, and ammonium bisulfate were measured to determine the neutralization of inhaled aerosol by ammonia in the lung. Exhaled ammonia was measured continuously by chemiluminescence after conversion to NO. Exhaled sulfates were monitored with a flame photometer after selective vaporization and absorption to separate H₂SO₄ and NH₄HSO₄.

Unneutralized sulfuric acid aerosol was measured in exhaled air during exposure to 10.5 mg/m³ H₂SO₄ with approximately 25% of the total exhaled sulfate measured as H₂SO₄. Comparable exposures conducted while blood ammonia was increased tenfold by continuous infusion of ammonium acetate showed no unneutralized H₂SO₄ in the exhaled air.

The pulmonary function parameters most sensitive to changes caused by aerosol exposure were closing volume, residual volume, and functional residual capacity. H₂SO₄ exposures with and without increased blood ammonia resulted in nearly identical dose related changes in closing volume suggesting no effect of blood ammonia on the pulmonary response to inhaled H₂SO₄ aerosols. Functional residual capacity and residual volume, however, were significantly lower following the 10.5 mg/m³ H₂SO₄ exposure compared with the 10.5 mg/m³ exposure with increased blood ammonia. In considering both of these results, increased blood ammonia may effect the pulmonary irritation caused by inhaled sulfuric acid aerosols. However, further experiments are required to substantiate this effect.

Five ppm NH₃ and 15 mg/m³ ammonium sulfate produced similar changes in pulmonary function. Tidal volume during exposure was increased and the slope of phase IV of the closing volume maneuver was steeper in both groups compared with controls. These changes were not seen during exposure to 7 mg/m³ ammonium sulfate.

These results indicate that neutralization of inhaled sulfuric acid aerosols can be increased by increasing blood ammonia concentrations. However, the increased neutralization does not seem to markedly effect the toxicity of inhaled acid aerosols.

This report was submitted in fulfillment of Contract No. 68-03-2665 by Battelle Memorial Institute under sponsorship of the U.S. Environmental Protection Agency. This report covers the period from April 18, 1978 to June 18, 1979 and work was completed as of May 18, 1979.

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SECTION 1

INTRODUCTION

Ammonia has been measured as a normal constituent of expired air in both dogs and humans (Jacquez et al., 1959; Kupprat, et al., 1976; Larson et al., 1977). Exhaled ammonia concentrations in tracheostomized dogs ranged from 2.3 to 6.4 nmoles/l alveolar air, while concentrations in humans ranged from 1.7 to 30 nmoles/l when breathing by mouth and 0.8 to 2.7 nmoles/l when breathing by nose. Larson et al. (1977) reported that the last airway traversed by respired gas before sampling appears to have the greatest effect on exhaled ammonia concentrations. Higher levels of ammonia were measured with the mouth traversed last, apparently from high concentrations of ammonia in the mouth due to bacterial action on saliva. Concentrations of ammonia measured in respired air from the nose and tracheobronchial tree appeared to reflect blood ammonia concentrations. Robin et al. (1959) reported that exhaled ammonia in dogs could be increased to 22.4 nmoles/l by increasing blood ammonia levels. This concentration represents a tenfold increase over the average concentration in exhaled air reported by Jacquez et al. (1959).

Ammonia has been shown to neutralize sulfuric acid aerosols both in the atmosphere and in the respiratory tracts of humans. Submicronic aerosols measured around Chicago (Cunningham and Johnson, 1976), St. Louis (Charlson et al., 1974) and in Western Sweden (Brosset et al., 1975) were primarily mixtures of sulfuric acid and neutralization products of sulfuric acid and ammonia (ammonium sulfate, $(\text{NH}_4)_2\text{SO}_4$, and ammonium bisulfate, NH_4HSO_4). Larson et al., (1977) reported neutralization of 0.6 mg/m³ inhaled sulfuric acid aerosols in humans, with an average ammonium to sulfate molar ratio in exhaled air greater than or equal to one.

Neutralization of inhaled sulfuric acid aerosols may be important in terms of acute and chronic toxicity. Amdur (1978) showed that the toxicity of inhaled sulfate compounds differed, depending on the associated cations. Ammonium sulfate aerosols were less toxic to guinea pigs than sulfuric acid aerosols of similar mass concentrations and particle size. In addition, Sim and Pattle (1957) found that symptoms of coughing, lacrimation, and rhinorrhea, caused by exposure of human subjects to a 39 mg/m³ H_2SO_4 aerosol, disappeared when ammonia was added to the aerosol. Since sulfuric acid may be neutralized by ammonia in the respiratory tract, it is important to determine the capacity of respiratory ammonia to neutralize inhaled sulfuric acid aerosols and to determine whether the toxicity of inhaled sulfuric acid aerosols is affected by neutralization with ammonia in the respiratory tract.

In this study, dogs were exposed to 0, 6, and 10.5 mg/m³ H₂SO₄ aerosols at normal ammonia levels, increased blood (endogenous) ammonia, and increased inhaled (exogenous) ammonia. Previous studies conducted in this laboratory on dogs exposed to 0, 1, 3.5, and 6.3 mg/m³ H₂SO₄ showed significant levels of unneutralized H₂SO₄ in exhaled air only at the highest acid level. Acute pulmonary effects were also greatest following exposure to the 6.3 mg/m³ aerosol. In this study, ammonia was added to the blood to determine if neutralization of H₂SO₄ in the lung was influenced by the blood ammonia concentration and to determine whether increased neutralization of acid aerosols in the lung affected acute toxicity. An additional group of exposures were conducted in which the dogs were exposed to an aerosol of NH₃ neutralized H₂SO₄ ((NH₄)₂SO₄) to assess the relative toxicity of neutralized sulfate vs. sulfuric acid alone.

SECTION 2

CONCLUSIONS

This study showed that, in dogs, increasing the ammonia concentration in blood tenfold (from 20 to 200 $\mu\text{moles/l}$) increased the neutralization of inhaled sulfuric acid aerosols to the point that no unneutralized H_2SO_4 was detected in exhaled air during exposure to 6 and 10.5 mg/m^3 H_2SO_4 . Without increased blood ammonia, unneutralized H_2SO_4 comprised approximately 25% of the total exhaled sulfate during exposure to 10.5 mg/m^3 H_2SO_4 .

The pulmonary function parameters most sensitive to changes caused by aerosol exposure were closing volume, residual volume, and functional residual capacity. H_2SO_4 exposures with and without increased blood ammonia resulted in nearly identical dose related changes in closing volume. Functional residual capacity and residual volume were significantly lower following the 10.5 mg/m^3 H_2SO_4 exposure compared with the comparable exposure with increased blood ammonia. In considering both of these results, increased blood ammonia may influence the pulmonary irritation caused by inhaled sulfuric acid aerosols. However, further experiments are required to substantiate this effect.

Five ppm NH_3 and 15 mg/m^3 $(\text{NH}_4)_2\text{SO}_4$ produced different responses from those measured during H_2SO_4 exposures. The increased tidal volumes during NH_3 and $(\text{NH}_4)_2\text{SO}_4$ exposures were probably caused by nasal irritation at least for the 5 ppm NH_3 exposure. The increased slope of Phase IV of the closing volume maneuver could also be due to reflex effects of nasal irritation since ammonia, due to its high solubility, probably did not penetrate beyond the nose. Although inhaled ammonia and ammonium sulfate were not innocuous, the dogs responses to these compounds were significantly different from their responses to H_2SO_4 aerosols indicating a different site or mechanism of effect.

SECTION 3

RECOMMENDATIONS

Experiments should be conducted to evaluate the site and time course of neutralization of inhaled sulfuric acid aerosols by ammonia in the lung. These studies should be conducted in experimental animals in which the degree of neutralization from the nose or mouth to the trachea can be evaluated at different flow rates and blood ammonia concentrations. The relationship between blood and exhaled ammonia or ammonia within the airways certainly requires further investigation. The retrograde catheter technique developed by Macklem and Mead (1967), to measure peripheral resistance in dogs, could also be used to evaluate the neutralization of acid aerosols in the lung from the trachea to a 2-mm airway.

Further studies on awake dogs inhaling acid aerosols may not be particularly useful because of the very high acid concentrations required to produce significant functional effects, and the limited information available in evaluating mixed exhaled air. These studies showed that the respiratory system of the dog is very effective in neutralizing inhaled sulfuric acid aerosols. The question now is to determine where, and how rapidly, this neutralization occurs.

SECTION 4

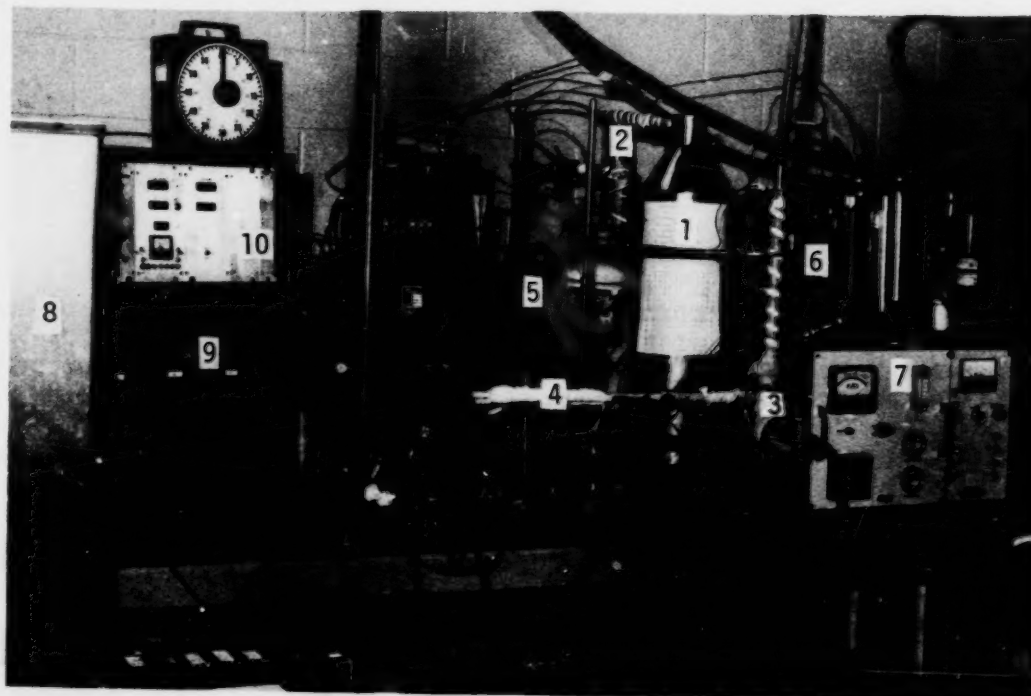
MATERIALS AND METHODS

GENERAL

Nine male beagles approximately 3 years old were exposed to 0, 6, and 12 mg/m³ sulfuric acid aerosols at normal ammonia levels, increased blood (endogenous) ammonia, and increased inhaled (exogenous) ammonia. Since the reactions between NH₃ and H₂SO₄ are very rapid, exposures to increased exogenous ammonia will be referred to as exposures to ammonium sulfate ((NH₄)₂SO₄) aerosols throughout this report. The apparatus for conducting these exposures is shown in Figure 1. The exposure groups and the order of exposures are shown in Table 1. All dogs were exposed to each condition at intervals of approximately one week. Parameters measured during exposure included respiration rate, tidal volume, minute volume, and concentrations of exhaled sulfuric acid, ammonium bisulfate, and gaseous ammonia. Pulmonary function and blood ammonia were evaluated before and after exposures. Data were analyzed using an unpaired t test making all possible comparisons between exposure groups. A paired t test was used to compare values before and after exposure. Statistical significance was established as $p < 0.05$.

AEROSOL GENERATION

Sulfuric acid aerosols were generated by vaporization/condensation of concentrated sulfuric acid using the system shown in Figure 2. High-purity N₂ was blown across a reservoir of sulfuric acid heated in a three-necked flask. This H₂SO₄-N₂ stream was diluted with a second N₂ stream before being combined with the main air supply to the aerosol chamber. The H₂SO₄-air stream traveled through a tube 30-cm-long, 1.2-cm in diameter, heated to about 220°C before reaching the exposure chamber. Mass concentration of the aerosol was controlled by adjusting the N₂ flow through the three-necked flask. Particle size could be adjusted by changing the temperature of the tube leading to the exposure chamber. Air flow to the chamber was 16 l/min and approximately 50% relative humidity. Before mixing with the H₂SO₄-N₂ stream, chamber air was passed through a Gelman, 47-mm, type AE glass fiber filter pad. The air was not scrubbed further since no appreciable ammonium sulfate was detected in sulfuric acid aerosols generated in this manner. During exposures, sulfuric acid concentration in the aerosol chamber was monitored with a Meloy SA160 flame photometric sulfur analyzer.



1. Aerosol Chamber
2. Aerosol Generation System
3. Dog Mask
4. H_2SO_4 Separation Apparatus
- 5 & 6. Meloy SA160-Sulfur Analyzers
7. Thermo Electron Model 12A NO/NO_x Analyzer
8. Grass Model 7 Polygraph
9. Temperature Regulators
10. Breath Counter and Tidal Volume Meter

Figure 1. Apparatus for exposing dogs to H_2SO_4 aerosols.

TABLE 1.
EXPOSURE GROUPS AND ORDER OF EXPOSURES

NH ₃ LEVEL	H ₂ SO ₄ CONCENTRATION (mg/m ³)	ORDER	CODE*
C	0	2	CC
C	6	4	C6
C	12	6	C12
EN	0	3	ENC
EN	6	1	EN6
EN	12	5	EN12
EX - 5 ppm NH ₃	0	7	EXC
EX	6 (NH ₄) ₂ SO ₄	9	EX6
EX	12 (NH ₄) ₂ SO ₄	8	EX12

C = Normal

EN = † Endogenous

EX = † Exogenous

* Abbreviations for each exposure group.

Mass concentration and particle size distribution of the aerosol were determined from filter pad and cascade impactor samples collected on days before and after dog exposures. Air flow through the sampling apparatus was controlled with critical orifices positioned between the apparatus and house vacuum. Flows were checked with a bubble flow meter before and after collecting samples. If flows differed by more than 5%, the samples were discarded. Gelman, type AE glass fiber filters were used in early sample collection. Because of high background sulfate levels and interference with our method for sulfate analysis caused by particles released from the filter pad, 0.45 µm HAWP filters (Millipore; Bedford, MA) and 0.25 µm FGLP filters (Millipore; Bedford, MA) were used

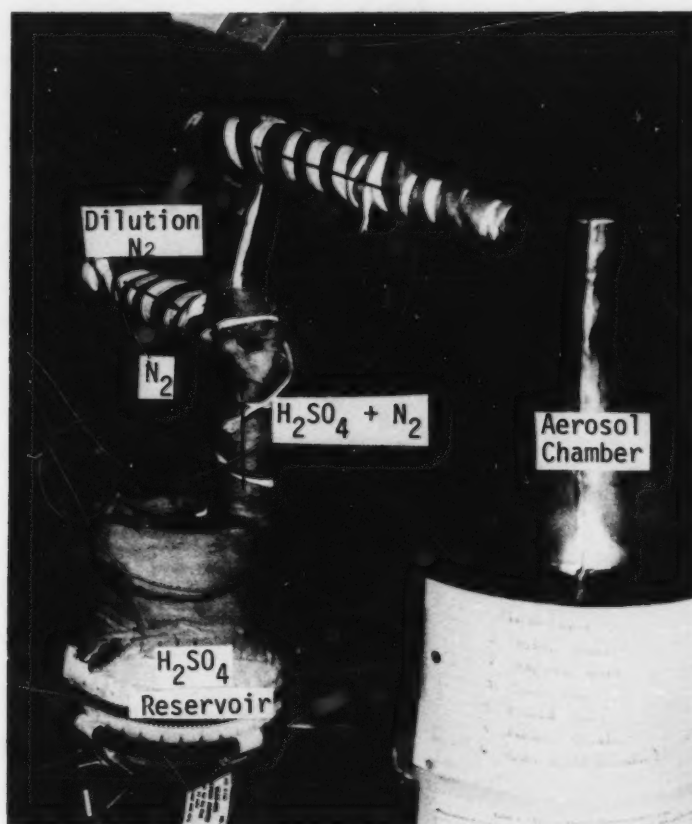


Figure 2. Sulfuric acid aerosol generation system.

for most sulfate analysis. Impactor samples were collected on a seven-stage Mercer impactor. Untreated, 22-mm glass cover slips were used to collect H_2SO_4 samples. Cover slips sprayed with Dow Corning Antifoam A to prevent particle bounce were used to collect $(\text{NH}_4)_2\text{SO}_4$ samples.

Sulfuric acid concentrations of impactor and filter pad samples were determined fluorometrically, based on the decreased fluorescence of a thorium-morin complex due to the precipitation of $\text{Th}(\text{SO}_4)_2$. Filter pads and glass cover slips were placed in vials containing 10 ml of distilled water and allowed to stand for at least 24 hours. Two ml of the sulfate sample, 0.5 ml of 10 $\mu\text{g/ml}$ Th^{4+} , 5 ml of 95% ethanol, and 2.5 ml of 0.002% morin were then added consecutively to a beaker. The pH was adjusted to 2.00 ± 0.05 with 1.0 N HCl. After 20 minutes, fluorescence was read on a spectrofluorometer, Mark I, Farrand Optical Co., at 420 nm excitation and

505 nm emission wavelengths. The working range of this procedure was from 0.5 to 100 $\mu\text{g H}_2\text{SO}_4/\text{ml}$, with a detection limit of 0.1 $\mu\text{g H}_2\text{SO}_4/\text{ml}$. Percent reproducibility ranged from 3 to 10%, depending on the sulfate concentration.

DOG EXPOSURES

Preceding exposures, dogs were treated with 0.75 mg/kg Acepromazine Maleate (Ayerst) injected intravenously as a tranquilizing agent. Each dog was then connected to the exposure apparatus for 30 minutes. He inhaled room air for 10 minutes and chamber air for the remaining 20 minutes through the system shown in Figure 3. The volume of air between the aerosol chamber and the dog mask was approximately 65 ml. Air flow was measured as the pressure drop across the venturi, and the flow signal was integrated and summed to determine total volume. Respiration rate was counted from pressure swings as air passed through the venturi. Mean minute volume (inhaled volume per minute) was calculated by dividing total volume by time. Mean tidal volume (inhaled volume per breath) was calculated by dividing total volume by total breaths. Respiration rate, tidal volume, and minute volume were recorded for 5-minute intervals throughout the exposures.

The mask used for dog exposures is shown in Figure 4. This mask was designed to minimize dead space and resistance to air flow by incorporating low-resistance, molded spiral valves (Hans Rudolph) for separating inspired from expired air into the body of the mask. The positions of exhaled sulfate and ammonia sample lines are also shown in Figure 4. During exposures, the end of the dog mask and the exhaled air line were heated to about 45°C to prevent condensation of water vapor.

EXHALED SULFATE ANALYSIS

Sulfuric acid and ammonium bisulfate concentrations in exhaled air were measured using the apparatus shown in Figure 3. This technique, developed by J. J. Huntzicker (1976), is based on the selective vaporization and absorption of sulfuric acid and ammonium bisulfate at different temperatures. Our apparatus was designed to first remove any gaseous ammonia by drawing the sample through a diffusion tube consisting of a 21-cm-long by 1.5-cm-diameter piece of flexible stainless steel tubing containing a cylinder of Gelman, type AE glass fiber filter material soaked in dilute phosphorous acid. The air sample was then divided into two streams and drawn through two parallel heated diffusion tubes consisting of 12-cm-long by 1.2-cm-diameter pieces of pyrex tubing containing cylinders of Gelman, type AE glass fiber filter material soaked in dilute sodium hydroxide solution. One tube was heated to 30°C for transmission of all exhaled sulfate particles. The other tube was heated to 120°C for vaporization and absorption of H_2SO_4 and transmission of ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$ or ammonium bisulfate $(\text{NH}_4\text{HSO}_4)$ particles.



1. Aerosol Chamber
2. Directional Valve to Room Air or Aerosol Chamber
3. Venturi
4. Exposure Mask
5. Exhaled Air-Reservoir
6. Ammonia Sampling Apparatus
7. Sulfate Sampling Apparatus

Figure 3. Connections of the exposure mask to the aerosol chamber and exhaled sampling apparatus.

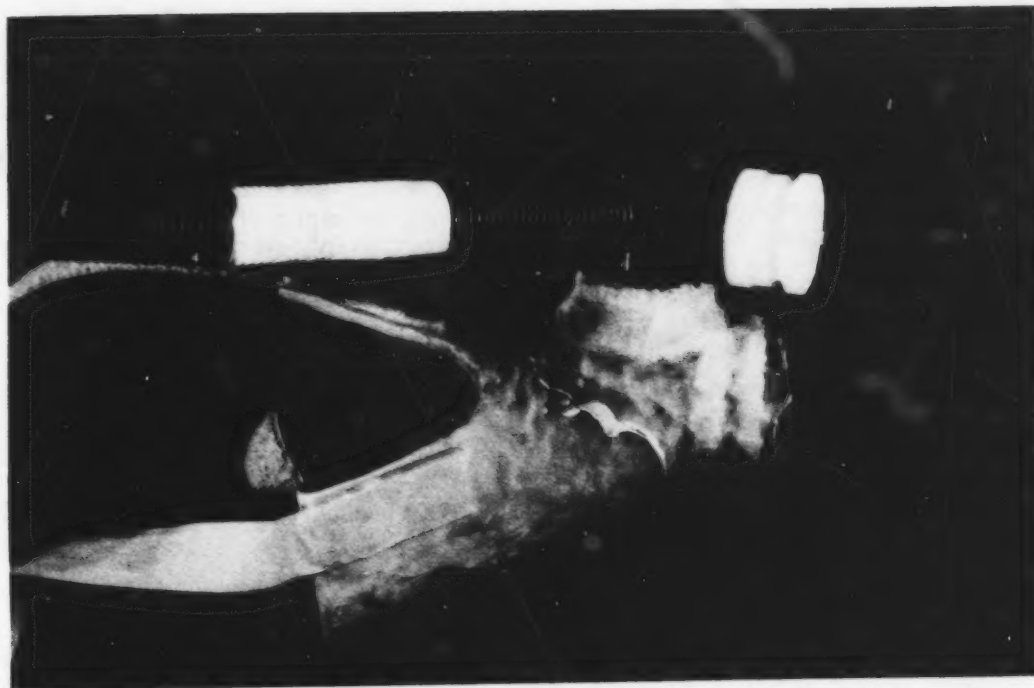


Figure 4. Mask used for dog exposures with exhaled ammonia and sulfate sampling lines attached.

Uniform heating was assured by surrounding the diffusion tubes with a 25-cm-length of 1.6-cm-diameter pyrex tubing wrapped with heater tape. Teflon tape wrapped at both ends of the 1.2-cm-diameter diffusion tubes assured a continuous air space between the 1.2 and 1.6-cm tubing and secured a Chromel-Alumel (type k) thermocouple in the air space between the tubes. Outputs of the thermocouples were displayed on a voltmeter and temperature was controlled by adjusting the voltage supplied to the heater tape. A third diffusion tube, heated to 220°C, was used initially to absorb H_2SO_4 and NH_4HSO_4 and transmit other less volatile sulfates (e.g., $(\text{Na})_2\text{SO}_4$). Since no material passed through this tube, we discontinued using the 220°C tube and concentrated on comparing total sulfate (30°C tube) with total sulfate minus H_2SO_4 (120°C tube).

During exposures, sulfate concentrations from the two diffusion tubes were measured alternately for 5 minute intervals. The tube not connected to the analyzer was exhausted at about 200 ml/min to prevent build-up of material. Total flow through the apparatus was about 400 ml/min.

The sulfur analyzer used to measure sulfur concentrations in exhaled air was calibrated following dog exposures. The aerosol generation system was adjusted so that the concentration of H_2SO_4 in the chamber, when measured through the 30°C tube, was similar to aerosol concentration normally measured in exhaled air. Sulfate concentrations in the aerosol chamber were then determined from samples collected on 0.45 μm HAWP millipore filter pads and analyzed fluorometrically.

EXHALED NH_3

The concentration of ammonia in exhaled air was measured with a Thermoelectron Model 12A $\text{NO}-\text{NO}_x$ analyzer containing a heated stainless steel catalyst to convert NH_3 to NO . We modified the instrument by replacing the sampling line with a 32-cm-length of P.E. 360 tubing, and by turning the temperature controller on the NH_3 converter from 750°C to 600°C . At 600°C the response time of the instrument was faster and output higher than at 750°C . The apparatus for withdrawing ammonia samples is shown in Figure 5. Because of the solubility of NH_3 in water, great care must be taken to prevent condensation of water vapor from exhaled

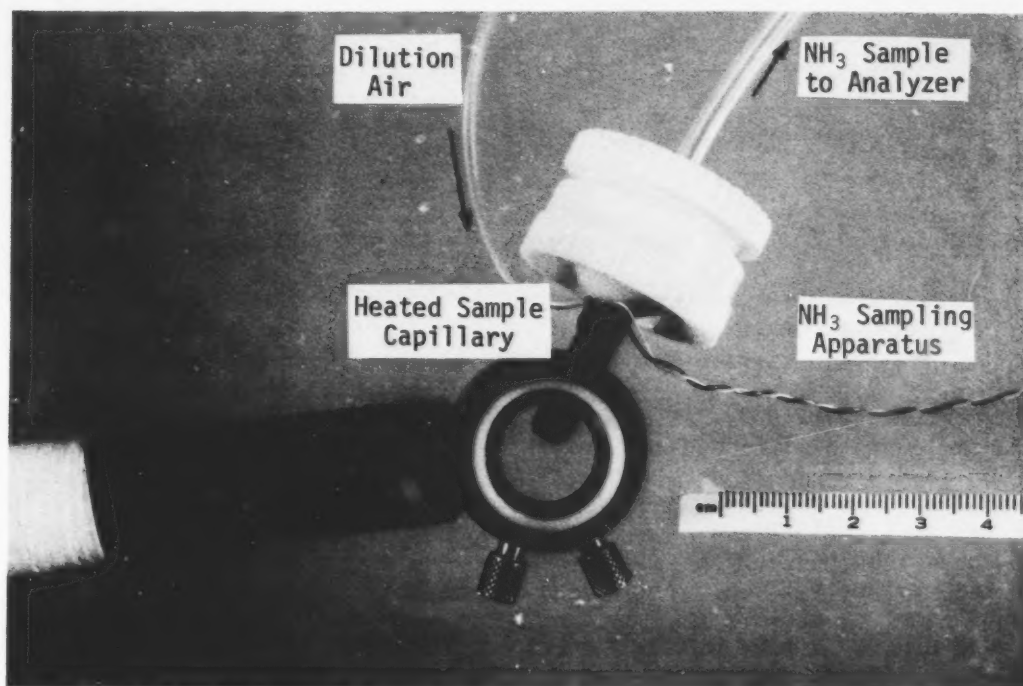


Figure 5. Position of sample lines for analysis of exhaled ammonia and sulfate concentrations.

breath. To prevent water condensation, the dog mask and exhaled air lines were heated to about 45°C . The ammonia sampling tip consisted of a 2.5-cm by 0.5-mm I.D. glass capillary wrapped with nichrome wire, surrounded by heat shrink tubing and heated to about 60°C . This sample tip was attached to a 25-mm filter holder. A stream of dry, ammonia free air was introduced into the filter holder through a hole next to the sample tip. This dry,

ammonia-free air diluted the exhaled gas sample by about 2.5:1 (clean air : exhaled gas). The combination of reduced pressure caused by the capillary restriction and dilution of the sample with clean air was sufficient to yield stable readings of exhaled ammonia concentrations. Clean dry air for dilution was obtained by passing compressed air through silica gel, chromium-trioxide-soaked glass fiber filter material to convert $\text{NO} \rightarrow \text{NO}_2$, activated charcoal to remove NO_2 , and a phosphorous-acid-soaked 47-mm filter pad to remove NH_3 . The experimental design called for measuring exhaled ammonia before and during exposure to H_2SO_4 . Initial experiments with Gelman, type AE 25-mm glass fiber filter pads soaked in dilute NaOH and placed in the filter holder showed good transmission of ammonia and removal of acid. However, bits of filter material released from the filter pad plugged the capillary tubes in the NH_3 analyzer, interfering with the response. In addition, although specifications on the instrument state that sulfur compounds do not interfere with analysis, particulate sulfates produced substantial positive readings. Because of these complicating factors, we measured exhaled ammonia concentrations only during the 10-minute period preceding acid exposure. In analyzing ammonia concentrations in exhaled breath, we found considerable background levels of NH_3 and NO in the room. Exhaled ammonia concentrations reported here reflect the difference between exhaled ammonia and background NH_3 plus NO_x levels in the room.

The ammonia analyzer was calibrated by diluting a standard gas containing 5 ppm NH_3 in nitrogen (Precision Gas Products, Santa Clara, CA) with clean, dry air. This gas mixture was sampled simultaneously by the Model 12A ammonia analyzer and by three glass bubblers (Ace Glass #752907 and 752915) filled with 10 ml of 0.01M H_2SO_4 , and arranged in parallel. Flow rates through the bubblers were approximately 350 ml/min. Preliminary experiments showed that all the ammonia was removed with a single bubbler. Samples were collected for one hour after which the ammonia concentrations in the bubblers were measured with a specific ion electrode (Orion Model 95-10).

BLOOD AMMONIA

Ammonia concentration in blood was measured using a "Blood Ammonia Test" kit (Hyland, Costa Mesa, CA). Jugular venous blood samples were collected before and immediately after exposure in heparinized syringes. During exposures with increased endogenous ammonia, samples were collected before beginning ammonia infusion, after 3 minutes of constant ammonia infusion, and immediately following exposure before discontinuing infusion. Samples were placed in a refrigerated centrifuge within two minutes of collection and spun at 2000 rpm for 20 minutes. The separated plasma was transferred to another sample tube, frozen in a dry-ice/water bath and stored at -70°C until analyzed. Initial samples were analyzed 24 hours after collection. However, repeated analysis of the same sample 24 hours and 4 days after collection showed no significant difference in ammonia concentrations. Therefore, some samples were stored for as long as 4 days before analysis.

INCREASED ENDOGENEOUS AMMONIA

Blood ammonia concentrations were increased by infusing a solution of 0.35 M ammonium acetate in sterile saline. The pH of the solution was adjusted from 7.0 to 7.4 by the addition of sodium bicarbonate. The solution was infused through a 20-g, 1.5-inch catheter needle (Becton-Dickinson) positioned in the radial vein using a Sage Instrument Model 01341 syringe pump. The initial infusion rate was set at approximately 5 ml/min for one minute to produce a rapid increase in blood ammonia. The infusion rate was then decreased to 0.6 ml/min during exposure. The dog was allowed to stabilize for about 5 minutes at the slower rate before beginning exposure.

INCREASED EXOGENOUS AMMONIA

The concentration of exogenous ammonia selected as being most comparable to the increased endogenous ammonia exposures was the concentration of ammonia which produced the same $\text{H}_2\text{SO}_4/\text{NH}_4\text{HSO}_4$ ratio in the aerosol chamber as measured in the expired air during exposure to $10.5 \text{ mg/m}^3 \text{ H}_2\text{SO}_4$, plus increased endogenous ammonia. Since no unneutralized H_2SO_4 was measured in exhaled breath during this exposure, exogenous ammonia (4950 ppm NH_3 in nitrogen, Matheson) was added to the main exposure air stream until no unneutralized H_2SO_4 was measured in the aerosol chamber at an initial acid concentration of 10.5 mg/m^3 . The ammonia concentration required to produce this neutralization was approximately 5 ppm.

PULMONARY FUNCTION

Pulmonary function measurements included pulmonary resistance and dynamic compliance on awake dogs, before and immediately (within 5 minutes) after exposure, and closing volume, lung volumes, quasi-static compliance, pulmonary resistance, and dynamic pulmonary compliance on pentothal-anesthetized dogs after exposure. Apparatus used for pulmonary function testing is shown in Figure 6. The mask, mouthpiece, and balloon for resistance and compliance measurements are shown in Figure 7. The esophageal balloon was positioned in the thoracic esophagus by advancing the balloon, stiffened by inserting a 0.6-mm cardiac catheter guide wire (Picker, Spokane, WA), through the mouth, down the esophagus, to the cardiac sphincter. The guide wire was then removed and the balloon withdrawn to a predetermined position which produced optimal respiratory oscillations with minimal cardiac artifact. Pulmonary resistance and dynamic compliance were determined by electronically manipulating signals of flow, volume, and transpulmonary pressure to achieve a horizontal line on a scope displaying transpulmonary pressure versus flow. This technique was developed by Miller and Simmons (1960) as a modification of the subtracter method of Mead and Whittenberger (1953). Because three of the nine dogs became very nervous during these measurements, and the time required to calm these dogs delayed additional function testing, measurements of awake resistance and compliance were made on the six calmer dogs.

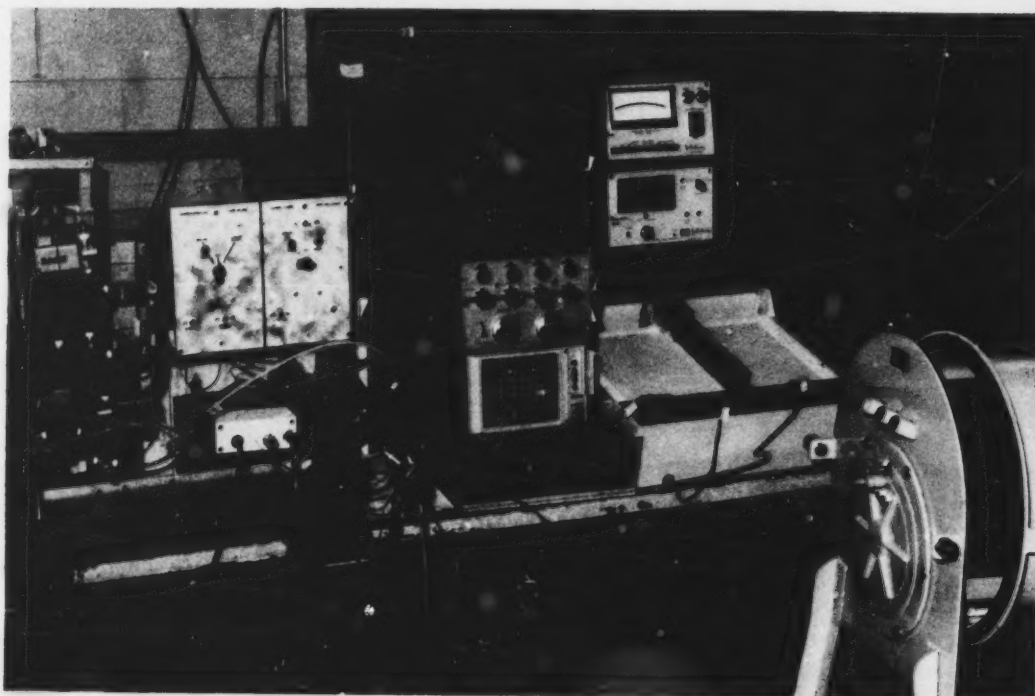


Figure 6. Pulmonary function testing apparatus.

Anesthetized tests were conducted with the dog lying prone in a sling. Quasi-static pressure vs. volume maneuvers, and closing volume maneuvers, were performed by inflating the lungs with either compressed air or oxygen and deflating with a vacuum. Flow was controlled at 75 ml/sec during both inflation and deflation by needle valves in the respiratory lines. Flow and volume were measured with a pneumotachometer. Nitrogen concentrations were measured with a fast-responding nitrogen analyzer (Hewlett-Packard Model 47302A). Transpulmonary pressure was measured as the difference between esophageal pressure and pressure at the end of the endotracheal tube, and transpulmonary pressure was used to control the level of inflation and deflation. Maximum inflation (total lung capacity), was defined as 30 cm H₂O transpulmonary pressure. Maximum deflation (residual volume), was defined as minus 10 cm H₂O transpulmonary pressure. At these pressures, relays were triggered which automatically stopped inflation or deflation.

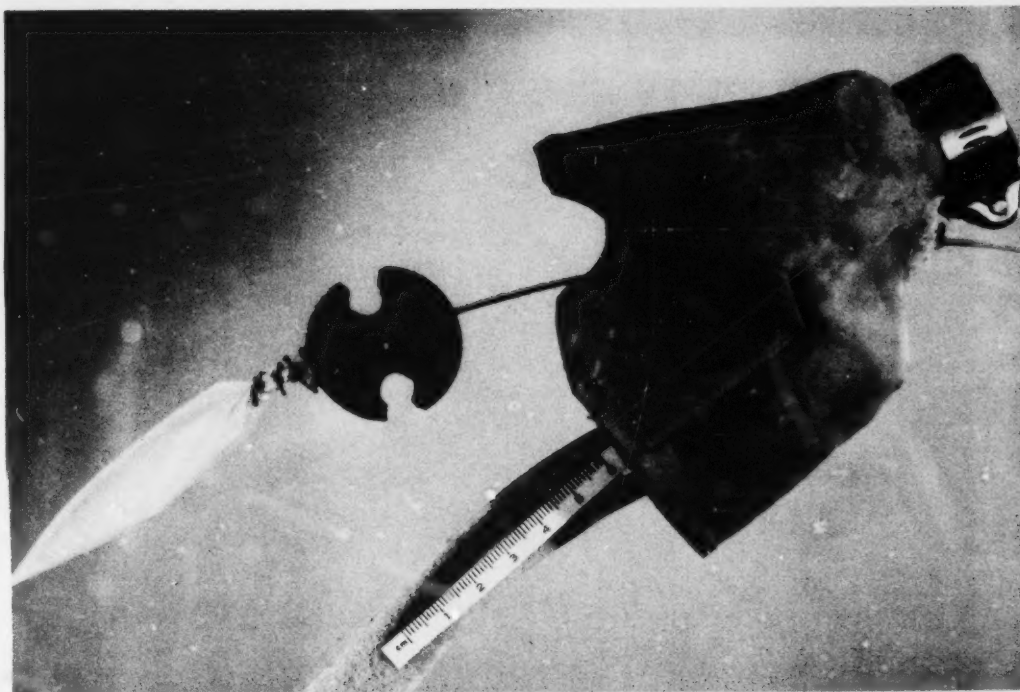


Figure 7. Mask, mouthpiece, and esophageal balloon for measuring pulmonary resistance and dynamic pulmonary compliance in awake dogs.

Vital capacity maneuvers were carried out by inflating the lungs from residual volume to total lung capacity and deflating to residual volume. During all tests, three vital capacity maneuvers were performed consecutively: during the first vital capacity, lungs were inflated with air to establish a constant volume history; during the second, lungs were again inflated with air, and transpulmonary pressure vs. volume curves were recorded for measurements of quasi-static compliance and vital capacity; during the third vital capacity, lungs were inflated with oxygen and closing volume curves were recorded. Following the third vital capacity, lungs were again inflated with air and the dogs were allowed to exhale passively through the pneumotachometer to functional residual capacity (FRC). Pulmonary resistance and dynamic compliance were measured with the dogs ventilated at 25 breaths per minute, 200 ml per breath, with a Harvard Model 613 ventilator. The FRC was measured using the method of DuBois, et al. (1956). Dogs were sealed in the plethysmograph, and plethysmograph pressure vs. mouth pressure was recorded as the dog attempted to inhale against a closed airway. The airway was closed at end exhalation by covering the airway opening. At least three FRC maneuvers were recorded on each dog, allowing three or four breaths between maneuvers. To facilitate analysis of pulmonary function

data on anesthetized animals, key points on each chart were digitized, and calculations were performed with a Hewlett-Packard 9825 calculator and peripheral digitizer.

SECTION 5

RESULTS AND DISCUSSIONS

Characteristics of the inhaled sulfuric acid aerosols are shown in Table 2. Mass concentrations for exposures specified at 6 mg/m³ were approximately 6.5 mg/m³ for the normal and increased endogenous ammonia exposures and 7.8 mg/m³ for the ammonium sulfate exposure. The particle size of the 6 mg/m³ aerosols was between 0.15 and 0.3 µm with a geometric standard deviation of 1.5. Mass concentrations for exposures specified at 12 mg/m³ were approximately 10.5 mg/m³ for the normal and increased endogenous ammonia exposures, and 15 mg/m³ for the ammonium sulfate exposure. The higher levels for the 6 and 12 mg/m³ ammonium sulfate exposures resulted from high readings for postexposure samples. The reasons for these higher values are not known, since generation conditions were the same before, during, and after exposure. The 7 and 15 mg/m³ ammonium sulfate aerosols were slightly larger than H₂SO₄ aerosols; however, all aerosols were less than 0.5-µm mass median aerodynamic diameter (MMAD).

EXHALED SULFATE

The sulfate concentrations measured in exhaled air are shown in Table 3. Total exhaled sulfate concentration (H₂SO₄ + (NH₄)₂SO₄) is measured through the 30°C sample tube and the ammonium bisulfate (NH₄HSO₄) concentration is measured through the 120°C sample tube. Preliminary experiments showed that all the exhaled sulfate was either H₂SO₄ or an ammonium salt of H₂SO₄. Throughout the exposures, no significant background was measured when the dogs breathed room air. Ammonium sulfate disassociates to NH₄HSO₄ plus NH₃ in the 120°C tube with the free NH₃ combining with previously unneutralized H₂SO₄ (Huntzicker, 1976). Therefore, a greater sulfate concentration measured through the 30°C tube, compared with the 120°C tube, indicates a molar ratio of SO₄ to NH₃ in exhaled air of greater than one.

The retention of inhaled aerosols measured in these studies was greater than 95% and is probably unrealistically high. Stauffer (1974) calculated a maximum deposition of 80% for 0.035 µm particles and results of the Task Group on Lung Dynamics (1966) predict 45% deposition for inert, non-hygroscopic aerosols of 0.2-µm MMAD, with a geometric standard deviation of 2. Because of the hygroscopic nature of H₂SO₄ and its tendency to conglomerate, retentions of greater than 45% are expected. Amdur, et al. (1952), measured a 77% average retention in 22 human subjects inhaling 1-µm H₂SO₄ aerosols. In addition, Swift et al. (1977) measured greater depositions of inhaled aerosols in dogs compared with humans under comparable conditions. Based

TABLE 2.
CHARACTERISTICS OF THE INHALED SULFURIC ACID AEROSOLS

Exposure Group*			Mass Conc. (mg/m ³)			Particle Size	
			$\bar{X}$	SD	N	MMAD*** (μm)	GSD****
C6	Pre	GF	6.54	0.35	3	0.19	1.4
	Post	-	-	-	-	0.22	1.3
C12	Pre	MP	9.5	0.8	3	0.21	1.7
	Post	FP	11.9	2.4	2	0.22	1.7
EN6	Pre	GF	6.52	0.33	2	0.16	1.8
	Post	GF	6.54	0.35	3	0.19	1.4
EN12	Pre	FP	10.4	0.8	3	0.19	1.7
	Post	MP	9.5	0.8	3	0.21	1.7
EX6	Pre	MP	6.78	1.2	4	-	-
	Post	MP	8.9	1.8	6	0.29	1.5
EX12	Pre	MP	11.3	2.9	6	0.41	2.0
	Post	MP	18.9	1.3	6	0.39	1.5

*First Symbol - NH₃

C = Normal

EN = † Endogenous

EX = † Exogenous

Second Symbol - Inhaled H₂SO₄ Concentration

6 = 6 mg/m³

12 = 12 mg/m³

**GF Gelman Type AE Glass Fiber

FP Millipore 0.2μm FGLP (Fluoropore)

MP Millipore 0.45μm HAWP (Millipore)

***MMAD - Mass Median Aerodynamic Diameter

****GSD - Geometric Standard Deviation

TABLE 3.
ANALYSIS OF EXHALED SULFATE
(nmoles SO₄/l Exhaled Air)

Exposure Group* (Inhaled Concentration)		30°C Tube		120°C Tube	
		10 Min	20 Min	15 Min	25 Min
C6 (66 nmoles/l)	$\bar{X}$	2.8 ⁶	2.8 ⁶	2.6 ⁶	2.5 ⁶
	SE	0.3	0.2	0.2	0.2
	N	6	6	6	6
C12 (109 nmoles/l)	$\bar{X}$	2.9 ^{6†}	3.0 ^{6†}	2.2 ^{6†}	2.3 ^{6†}
	SE	0.2	0.2	0.2	0.2
	N	9	9	9	9
EN6 (66 nmoles/l)	$\bar{X}$	2.4 ⁶	2.4 ⁶	2.4 ⁶	2.5 ⁶
	SE	0.2	0.2	0.2	0.2
	N	8	5	8	5
EN12 (101 nmoles/l)	$\bar{X}$	2.8 ⁶	2.9 ⁶	2.7 ⁶	2.8 ⁶
	SE	0.3	0.2	0.2	0.2
	N	9	9	9	9
EX6 (80 nmoles/l)	$\bar{X}$	2.3 ^{6††}	2.3 ^{6††}	2.6 ^{6††}	2.9 ^{6††}
	SE	0.4	0.4	0.4	0.6
	N	9	9	9	9
EX12 (154 nmoles/l)	$\bar{X}$	3.9 ^{(1-5)††}	4.3 ^{(1-5)††}	5.2 ^{(1-5)††}	5.5 ^{(1-5)††}
	SE	0.4	0.5	0.6	0.6
	N	9	9	9	9

*First Symbol - NH₃

C = Normal

EN = † Endogenous

EX = † Exogenous

Second Symbol - Inhaled H₂SO₄ Concentration

6 = 6 mg/m³

12 = 12 mg/m³

†Output of 30°C tube > 120°C tube (p < 0.05; paired t test)

††Output of 30°C tube < 120°C tube (p < 0.05; paired t test)

Superscripts indicate groups which are significantly different (p < 0.05; unpaired t test).

1 = C6

4 = EN12

2 = C12

5 = EX6

3 = EN6

6 = EX12

on these factors, deposition of between 80 and 90% of the inhaled aerosol in the respiratory tract is possible.

During exposure to 15 mg/m³ ammonium sulfate, the total exhaled sulfate concentration was higher ($p < 0.05$, unpaired t test) than in all other groups. The lack of any significant difference between exhaled sulfate concentrations during exposures to 6 and 10.5 mg/m³ H₂SO₄ may be caused by the very high retention of inhaled aerosol masking small differences in the exhaled concentration.

The presence of unneutralized sulfuric acid in the exhaled air was determined by comparing the sulfate concentrations measured through the 30° and 120°C sample tubes, with a higher output from the 30°C tube indicating unneutralized H₂SO₄. Significant ($p < 0.05$, paired t test) levels of unneutralized H₂SO₄ were measured only during exposure to 10.5 mg/m³ H₂SO₄. The unneutralized acid comprised about 25% of the total exhaled sulfate concentration. During ammonium acetate infusions, no unneutralized H₂SO₄ was measured in exhaled air.

In our previous study on the neutralization of inhaled sulfuric acid, we found significant unneutralized acid in the exhaled air during exposure to 6.3 mg/m³ H₂SO₄. Although in the study reported here the outputs of the 30°C tube tended to be higher than the 120°C tube during exposure to 6 mg/m³ H₂SO₄, the difference between the two sample tubes was not significant, based on a paired t test. Two factors may contribute to the difference between these studies. In the first study, apparatus dead space was greater, contributing a greater concentration of uninhaled aerosol to exhaled air. In addition, dogs were not tranquilized in the first study; their more rapid, shallow breathing (caused by general excitement and by the irritating effects of the acid) could decrease the time for neutralization in the respiratory tract. Although the reaction between NH₃ and H₂SO₄ is practically instantaneous, the ammonia concentration in the lung is so low that ammonia must continually diffuse from the respiratory surface into the airways to produce any substantial neutralization. With the increased respiration rates, less time would be available for diffusion.

Table 3 shows that during ammonium sulfate exposures, the sulfate concentration measured through the 120°C tube was greater than through the 30°C tube ($p < 0.05$, paired t test). The reason for this difference is not known. When ammonium sulfate aerosols were sampled from the aerosol chamber, the output of both tubes was the same.

BLOOD AMMONIA

Blood ammonia concentrations measured before and after exposure are shown in Table 4 and averaged 21 μ moles/l pre-exposure. Minor differences were measured among several of the pre-exposure groups reflecting day-to-day variations. However, these differences were small compared with the tenfold increase in blood ammonia during ammonium acetate infusion. The infusion schedule used in this study produced a reproducible increase in blood ammonia which did not change significantly during the 30 minute exposure.

TABLE 4.
BLOOD AMMONIA CONCENTRATION (μ MOLES/L BLOOD)

Exposure Group*		Pre-Infusion	Pre-Exposure	Postexposure	Significant** Difference Pre-Post
CC	$\bar{X}$	--	23	27	ND
	SE	--	1.8	2.2	
	N	--	9	9	
C6	$\bar{X}$	--	21	27	p < 0.05
	SE	--	1.6	1.6	
	N	--	9	5	
C12	$\bar{X}$	--	19	24	ND
	SE	--	1.7	2.3	
	N	--	9	6	
ENC	$\bar{X}$	25	208	240	ND
	SE	1.6	26	22	
	N	9	8	8	
EN6	$\bar{X}$	23	189	198	ND
	SE	1.5	39	30	
	N	9	9	9	
EN12	$\bar{X}$	19	214	204	ND
	SE	2.1	24	16	
	N	9	9	9	
EXC	$\bar{X}$	--	21	26	p < 0.05
	SE	--	1.4	2.1	
	N	--	9	9	
EX6	$\bar{X}$	--	16	22	p < 0.05
	SE	--	1.0	2.6	
	N	--	9	9	
EX12	$\bar{X}$	--	16	20	ND
	SE	--	0.5	1.3	
	N	--	9	9	

*First Symbol - NH_3

C = Normal

EN = $\uparrow$ Endogenous

EX = $\uparrow$ Exogenous

Second Symbol - Inhaled H_2SO_4 Concentration

C - 0 mg/m^3

6 = 6 mg/m^3

12 = 12 mg/m^3

**Paired t test comparing pre- and postexposure values for each dog.

ND = Not significantly different

During exposure to $6 \text{ mg/m}^3 \text{ H}_2\text{SO}_4$, 5 ppm NH_3 , and $6 \text{ mg/m}^3 (\text{NH}_4)_2\text{SO}_4$ blood ammonia was significantly higher ($p < 0.05$, paired t test) post-exposure than pre-exposure. The increased ammonia postexposure in dogs exposed to 5 ppm NH_3 and $6 \text{ mg/m}^3 (\text{NH}_4)_2\text{SO}_4$ may be the result of inhalation of ammonia. However, factors responsible for the increased ammonia following exposure to $6 \text{ mg/m}^3 \text{ H}_2\text{SO}_4$ are not known. This increase could be related to the exposure since ammonia also tended to increase following exposure to air only and $10.5 \text{ mg/m}^3 \text{ H}_2\text{SO}_4$.

EXHALED AMMONIA

Ammonia concentrations in exhaled air were measured during the 10 minute period before aerosol exposure and mean values for the nine exposures are shown in Table 5. Figure 8 is a record of ammonia exhaled from a dog preceding exposure to $6 \text{ mg/m}^3 \text{ H}_2\text{SO}_4$. This record shows that during the 10 minute sampling time, the measured concentration of exhaled ammonia

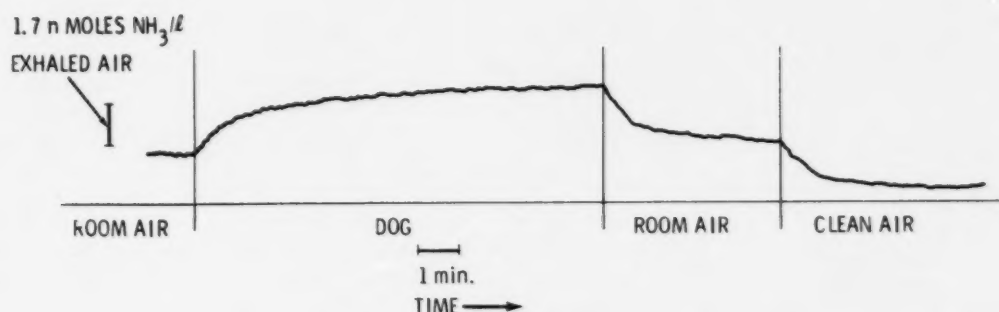


Figure 8. Record of exhaled ammonia from a dog with normal blood ammonia.

increased to a relatively constant level which was about twice the background concentration measured with the sample tip drawing in room air. The background $\text{NH}_3 + \text{NO}_x$ level in the room was equivalent to about $1.2 \text{ nmoles NH}_3/\text{l}$. Ammonia concentrations after subtracting background and at normal levels of blood ammonia were approximately 2.0 nmoles/l . These values agree well with values of approximately 1.5 nmoles/l measured by Larson et al. (1977), in humans exhaling through the nose and 2.5 nmoles/l measured by Larson et al. (1979) in tracheal air of dogs. During ammonia infusions, exhaled ammonia was generally higher than values with normal blood ammonia although the increase was not as great as might be expected from the tenfold increase in blood ammonia.

TABLE 5.
AMMONIA CONCENTRATION IN EXHALED AIR (nmoles/l)

Exposure Group*	$\bar{X}$	SE	N
CC	2.01	0.31	8
C6	1.25	0.08	4
C12	-	-	-
ENC	1.81**	0.18	7
EN6	2.11**	0.37	7
EN12	2.90**	0.30	9
EXC	2.57	0.17	6
EX6	1.43	0.13	8
EX12	1.96	0.30	8

*First Symbol - NH_3

C = Normal

EN = $\uparrow$ Endogenous

EX = $\uparrow$ Exogenous

Second Symbol - Inhaled H_2SO_4 Concentration

C = 0 mg/m^3

6 = 6 mg/m^3

12 = 12 mg/m^3

**Exhaled ammonia did not increase significantly during ammonia infusions which increased blood ammonia tenfold.

The reason for the insignificant change in exhaled ammonia with a tenfold increase in blood ammonia is not known. Robin et al. (1959) measured exhaled ammonia concentrations of 22 nmoles/l (approximately ten times the values reported here) in dogs during ammonium acetate infusions which increased blood ammonia to 500 $\mu\text{moles/l}$. This work suggests that the increase in exhaled ammonia is relatively less than the increase in blood ammonia. However, the relationship between blood and exhaled ammonia requires further investigation. Because of the complex chemistry of blood and the extremely low concentrations of ammonia present in blood, there may not be a one-to-one relationship between blood and exhaled ammonia.

Pulmonary Function

Breathing patterns (tidal volumes, respiration rates, and minute volumes) were measured before and during exposures and the results are shown in Tables 6-8. After breathing through the exposure apparatus for 5 minutes, respiration was fairly well stabilized and breathing patterns during the last 5 minutes pre-exposure are reported here. Values during exposure were averaged for the first 10 minutes and last 10 minutes of exposure. Most of the significant differences among exposure groups were measured as differences in tidal volume, Table 6.

Breathing patterns pre-exposure were not significantly different among any of the exposure groups. Tidal volumes during exposure to $6 \text{ mg/m}^3 \text{ H}_2\text{SO}_4$ plus increased endogenous ammonia (EN6) were significantly lower ($p < 0.05$, unpaired t test) than during several other exposures. EN6 was the first exposure and although the dogs were tranquilized and had previously been conditioned to breath through the exposure apparatus, they seemed more apprehensive than during later exposures. This anxiety was probably responsible for the lower tidal volumes.

Tidal volumes during exposure to 5 ppm NH_3 (20-30 min) were significantly higher than during the control exposure (CC). Five ppm ammonia is at the odor threshold of ammonia for humans (Mangold, 1971). With the dogs more sensitive olfactory system, this concentration of ammonia was probably readily detected causing increased tidal volumes. Ammonium sulfate is generally considered to be much less irritating to the respiratory tract than sulfuric acid (Amdur, 1970; Sim and Pattle, 1957). However, tidal volumes showed similar changes during exposure to $10.5 \text{ mg/m}^3 \text{ H}_2\text{SO}_4$ and $15 \text{ mg/m}^3 (\text{NH}_4)_2\text{SO}_4$. During both exposures, tidal volumes tended to increase while tidal volumes during the control exposure decreased significantly compared with pre-exposure values ($p < 0.05$; paired t test). The significant increase during exposure to $15 \text{ mg/m}^3 (\text{NH}_4)_2\text{SO}_4$ compared with control but not during exposure to $10.5 \text{ mg/m}^3 \text{ H}_2\text{SO}_4$ compared with control is probably due to the higher concentration of the ammonium sulfate aerosols. These results indicate that ammonium sulfate and sulfuric acid aerosols have similar effects on breathing pattern under these exposure conditions.

The mechanical properties of the lungs, shown in Tables 9 and 10, were not affected by acid exposure. A decrease in dynamic compliance following exposure to $10.5 \text{ mg/m}^3 \text{ H}_2\text{SO}_4$ plus increased endogenous ammonia compared with increased endogenous ammonia alone was the only significant change associated with aerosol exposure. Significant day-to-day variations were detected in measurements of awake resistance and compliance. However, these changes were not related to exposure and to avoid confusion they were not marked on the tables.

TABLE 6.

PULMONARY FUNCTION DATA: TIDAL VOLUME BEFORE AND DURING EXPOSURE

		PRE-EXPOSURE	DURING EXPOSURE	
		5-10 Min	10-20 Min	20-30 Min
Exposure Group*		Tidal Volume (ml)	Tidal Volume (ml)	Tidal Volume (ml)
CC	$\bar{X}$	165 [†]	153 ⁹	146 ^{7,9,†}
	SE	7.6	7.5	7.9
	N	8	8	9
C6	$\bar{X}$	160	152	139
	SE	13.1	18.5	21.8
	N	6	6	5
C12	$\bar{X}$	159	167	172 ⁵
	SE	10.4	13.1	12.5
	N	9	9	9
ENC	$\bar{X}$	174	166 ⁵	168 ⁵
	SE	7.9	6.2	7.4
	N	8	8	8
EN6	$\bar{X}$	153	138 ^{4,6,7,9}	132 ^{3,4,6,7,9}
	SE	7.3	4.5	6.8
	N	5	5	4
EN12	$\bar{X}$	162	169 ⁵	161 ⁵
	SE	11.5	9.5	8.7
	N	8	9	9
EXC	$\bar{X}$	174	175	187 ^{1,5}
	SE	13.8	12.3	14.1
	N	9	9	9
EX6	$\bar{X}$	160	159	163
	SE	13.0	14.8	16.7
	N	9	9	9
EX12	$\bar{X}$	174	192 ^{1,5}	185 ^{1,5}
	SE	15.7	14.4	15.7
	N	8	9	9

*First Symbol - NH₃

C = Normal

EN = † Endogenous

EX = † Exogenous

Second Symbol - Inhaled H₂SO₄ ConcentrationC = 0 mg/m³6 = 6 mg/m³12 = 12 mg/m³

†Significant Difference Pre vs. During (p < 0.05; paired t test)

Statistical Significance: Superscripts indicate groups which are significantly different (p < 0.05; unpaired t test)

1 = CC

4 = ENC

7 = EXC

2 = C6

5 = EN6

8 = EX6

3 = C12

6 = EN12

9 = EX12

TABLE 7.

PULMONARY FUNCTION DATA: RESPIRATION RATE BEFORE AND DURING EXPOSURE

Exposure Group*		Pre-Exposure	DURING EXPOSURE	
		5-10 Min	10-20 Min	20-30 Min
		Respiration Rate (BPM)**	Respiration Rate (BPM)**	Respiration Rate (BPM)**
CC	$\bar{X}$	17.1	16.1	17.6
	SE	1.9	1.3	1.4
	N	9	9	9
C6	$\bar{X}$	17.4	17.1	15.5
	SE	1.7	1.8	1.7
	N	6	6	5
C12	$\bar{X}$	15.8	15.5	14.4
	SE	1.6	1.2	1.3
	N	9	9	9
ENC	$\bar{X}$	16.6	17.8	18.0
	SE	1.8	1.8	2.0
	N	8	8	8
EN6	$\bar{X}$	16.3	18.4	16.0
	SE	2.0	1.9	1.2
	N	5	5	4
EN12	$\bar{X}$	15.7	15.9	15.3
	SE	1.5	1.2	0.9
	N	8	9	9
EXC	$\bar{X}$	15.8	14.8	14.4
	SE	2.7	2.3	1.9
	N	9	9	9
EX6	$\bar{X}$	17.1	15.7	14.9
	SE	3.5	2.5	2.8
	N	9	9	8
EX12	$\bar{X}$	17.9	15.1	15.6
	SE	2.2	1.8	1.7
	N	8	9	9

*First Symbol - NH_3

C = Normal

EN = + Endogenous

EX = + Exogenous

Second Symbol - Inhaled H_2SO_4 ConcentrationC = 0 mg/m^3 6 = 6 mg/m^3 12 = 12 mg/m^2

**BPM = Breaths/minute

TABLE 8.

PULMONARY FUNCTION DATA: MINUTE VOLUME BEFORE AND DURING EXPOSURE

Exposure Group*		Pre-Exposure	DURING EXPOSURE	
		5-10 Min	10-20 Min	20-30 Min
		Minute Volume (ml/min)	Minute Volume (ml/min)	Minute Volume (ml/min)
CC	$\bar{X}$	2860	2519	2531
	SE	250	171	220
	N	8	8	9
C6	$\bar{X}$	2843	2673	2185
	SE	446	485	493
	N	6	6	5
C12	$\bar{X}$	2392	2464	2391
	SE	124	121	179
	N	9	9	9
ENC	$\bar{X}$	2867	2951	2969 ^{5,8}
	SE	292	312	281
	N	8	8	8
EN6	$\bar{X}$	2142	2515	2099 ^{4,9}
	SE	595	250	91
	N	5	5	4
EN12	$\bar{X}$	2421	2611	2444
	SE	125	155	155
	N	8	9	9
EXC	$\bar{X}$	2526	2393	2491
	SE	227	167	159
	N	9	9	9
EX6	$\bar{X}$	2487	2264	2160 ⁴
	SE	275	177	193
	N	9	9	8
EX12	$\bar{X}$	2920	2801	2765 ⁵
	SE	229	186	218
	N	8	9	9

*First Symbol -

NH₃

C = Normal

EN = † Endogenous

EX = † Exogenous

Second Symbol -

Inhaled H₂SO₄ ConcentrationC = 0 mg/m³6 = 6 mg/m³12 = 12 mg/m³

Statistical Significance: Superscripts indicate groups which are significantly different (p < 0.05; unpaired t test)

1 = CC

3 = C12

5 = EN6

7 = EXC

9 = EX12

2 = C6

4 = ENC

6 = EN12

8 = EX6

TABLE 9.
PULMONARY FUNCTION DATA: PULMONARY RESISTANCE

Exposure Group*		Pulmonary Resistance (cm H ₂ O/l/sec)		Anesthetized
		Pre-Exposure	Postexposure	
CC	$\bar{X}$	31.0	28.7	2.5
	SE	3.5	3.6	0.1
	N	8	8	9
C6	$\bar{X}$	23.4	34.4	2.6
	SE	4.5	10.4	0.1
	N	4	3	6
C12	$\bar{X}$	22.4	29.8	2.6
	SE	2.5	5.0	0.2
	N	5	5	9
ENC	$\bar{X}$	28.5	28.0	2.3
	SE	3.3	2.1	0.2
	N	7	6	8
EN6	$\bar{X}$	---	---	2.6
	SE	---	---	0.2
	N	---	---	7
EN12	$\bar{X}$	19.4	22.7	2.5
	SE	3.7	2.3	0.2
	N	5	6	9
EXC	$\bar{X}$	22.4	30.0	2.5
	SE	1.9	4.1	0.1
	N	6	5	9
EX6	$\bar{X}$	27.3	24.7	2.5
	SE	4.6	2.9	0.1
	N	6	4	9
EX12	$\bar{X}$	29.3	30.4	2.5
	SE	4.7	2.4	0.1
	N	6	6	9

* First Symbol -

NH₃

C = Normal

EN = ↑ Endogenous

EX = ↑ Exogenous

Second Symbol -

Inhaled H₂SO₄ Concentration

C = 0 mg/m³

6 = 0 mg/m³

12 = 12 mg/m³

TABLE 10.

PULMONARY FUNCTION DATA: PULMONARY COMPLIANCE

Exposure Group*		Dynamic Pulmonary Compliance (ml/cm H ₂ O)			Quasi-Static Pulmonary Compliance (ml/cm H ₂ O)
		Pre-Exposure	Postexposure	Anesthetized	
CC	$\bar{X}$	49.8	45.0	87.9	107
	SE	5.8	3.7	6.1	6.8
	N	8	8	9	9
C6	$\bar{X}$	44.2	56.8	85.3	98
	SE	10.5	20.6	8.3	5.6
	N	4	3	6	6
C12	$\bar{X}$	59.7	47.6	83.7	109
	SE	6.6	3.2	4.4	8.3
	N	5	5	9	9
ENC	$\bar{X}$	37.6	46.8	98.5 ⁶	107
	SE	3.6	8.4	7.3	7.8
	N	7	6	8	8
EN6	$\bar{X}$	---	---	116	120
	SE	---	---	18.3	12.0
	N	---	---	7	8
EN12	$\bar{X}$	62.1	52.6	78.8 ⁴	98
	SE	3.3	7.0	5.8	7.1
	N	5	6	9	9
EXC	$\bar{X}$	69.1	60.1	88.5	101
	SE	9.5	10.5	7.8	6.8
	N	6	6	9	9
EX6	$\bar{X}$	42.9	64.1	89.6	98
	SE	8.9	18.8	7.3	6.3
	N	6	4	9	9
EX12	$\bar{X}$	46.4	43.8	96.3	107
	SE	9.2	8.7	7.8	7.5
	N	6	6	9	9

* First Symbol -

NH₃

C = Normal

EN = ↑ Endogenous

EX = ↑ Exogenous

Second Symbol -

Inhaled H₂SO₄ ConcentrationC = 0 mg/m³6 = 6 mg/m³12 = 12 mg/m³

Statistical Significance: Superscripts indicate groups which are significantly different ($p < 0.05$; unpaired t test)

1 = CC

3 = C12

5 = EN6

7 = EXC

9 = EX12

2 = C6

4 = ENC

6 = EN12

8 = EX6

Differences were also measured between values for awake and anesthetized resistance and compliance. These differences were expected due to the effects of anesthesia and endotracheal intubation. They do not indicate any effects of aerosol exposure.

Measurements of closing volume and lung volumes (Tables 11 and 12) appeared to be most useful in comparing the toxicity of different exposures. H_2SO_4 exposures with and without increased blood ammonia resulted in nearly identical dose related changes in closing volume (Figure 9). Closing volumes measured following the 10.5 mg/m^3 exposures with and without increased blood ammonia were both significantly higher than following control and 5 ppm NH_3 exposures. If changes in closing volume are related to an acute toxic effect of aerosol exposure then the similar dose response relationships between normal and increased blood ammonia suggest little effect of blood ammonia on the response of the lung to inhaled sulfuric acid aerosols.

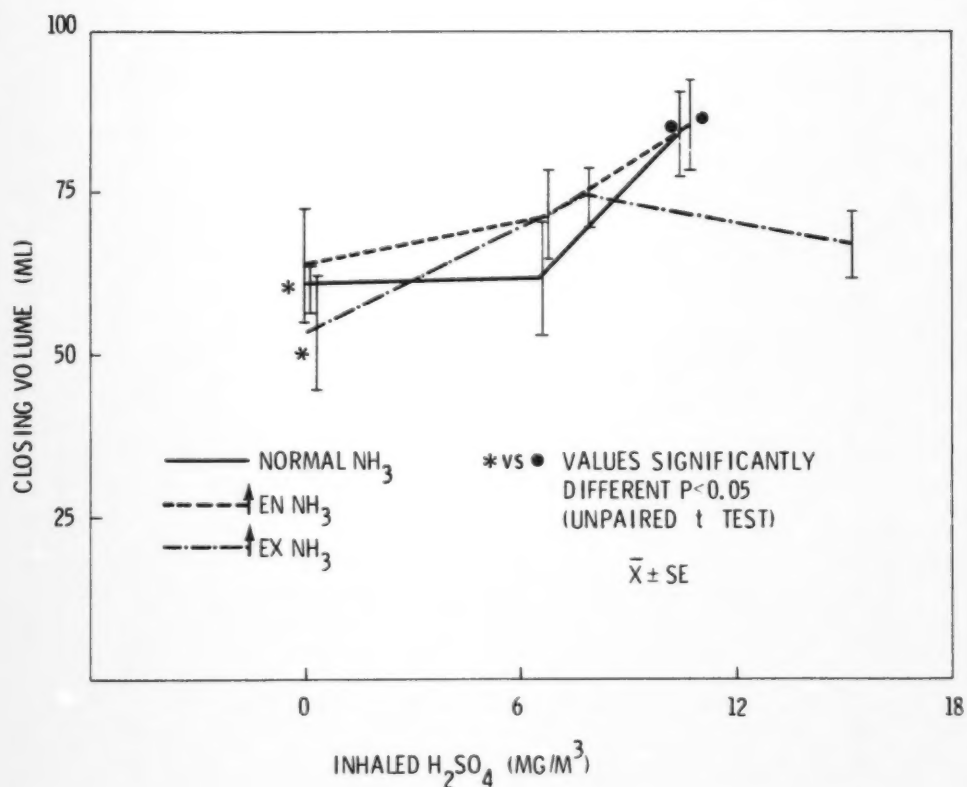


Figure 9. H_2SO_4 concentration vs. closing volume; normal, increased endogenous, and increased exogenous ammonia.

TABLE 11.
PULMONARY FUNCTION DATA: MEASUREMENTS FROM CLOSING VOLUME MANEUVERS

Exposure Group*	Anatomic Dead Space (ml)	M-3 (% N ₂ /ℓ)	M-4 (% N ₂ /ℓ)	Closing Volume (ml)	Closing Volume/Vital Capacity (%)	Closing Capacity (ml)	Closing Capacity/Total Lung Capacity (%)
CC	125	0.62	4.71 ^{7,9}	60.7 ^{3,6}	5.6 ⁶	443	30
	SE	0.05	0.34	4.7	0.5	58	2.7
	N	9	9	9	9	8	8
C6	141	0.57 ⁹	4.97	61.8	5.2	448	29
	SE	0.05	1.28	16.7	1.4	41	1.6
	N	6	5	6	6	6	6
C12	124	0.67	4.10 ^{7,9}	84.0 ^{1,7}	7.3 ⁷	358 ⁶	25 ^{6,8}
	SE	0.04	0.24	8.0	0.7	26	1.4
	N	9	9	9	9	9	9
ENC	128	0.64	5.12	64.0	5.8	445	30
	SE	0.04	0.63	12.6	1.1	34	2.2
	N	8	7	8	8	8	8
EN6	137	0.70	4.24 ^{7,9}	70.3	6.0	407	27
	SE	0.04	0.38	8.6	0.6	42	1.8
	N	8	8	8	8	6	6
EN12	129	0.64	4.04 ^{7,9}	85.1 ^{1,7}	7.5 ^{1,7}	461 ^{3,7}	31 ³
	SE	0.03	0.31	8.8	0.6	31	2.0
	N	9	9	9	9	9	9
EXC	125	0.70	6.73 ^{1,3,5,6}	53.6 ^{3,6}	4.8 ^{3,6,8}	374 ⁶	26 ⁸
	SE	0.09	0.41	8.4	0.7	24	0.8
	N	9	8	9	9	9	9
EX6	121	0.79	5.07	74.6	7.0 ⁷	419	29 ^{3,7}
	SE	0.08	0.51	5.7	0.6	22	1.1
	N	9	9	9	9	9	9
EX12	127	0.71 ²	6.48 ^{1,3,5,6}	67.4	6.1	389	27
	SE	0.03	0.66	7.4	0.7	22	0.9
	N	9	9	9	9	9	9

* First Symbol - NH₃
C = Normal
EN = † Endogenous
EX = † Exogenous

Second Symbol - Inhaled H₂SO₄ Concentration
C = 0 mg/m³
6 = 6 mg/m³
12 = 12 mg/m³

Statistical Significance: Superscripts indicate groups which are significantly different (p < 0.05; unpaired t test)
1 = CC 2 = C6 3 = C12 4 = ENC 5 = EN6 6 = EN12 7 = EXC 8 = EX6 9 = EX12

TABLE 12.
PULMONARY FUNCTION DATA: LUNG VOLUMES

Exposure Group*		Residual Volume (ml)	Functional Residual Capacity (ml)	Vital Capacity (ml)	Total Lung Capacity (ml)
CC	$\bar{X}$	383	589	1100	1455
	SE	58	66	43	81
	N	8	8	9	8
C6	$\bar{X}$	386 ³	600	1167	1553
	SE	33	48	41	64
	N	6	6	6	6
C12	$\bar{X}$	275 ^{2,4,6,8}	518 ⁶	1140	1415
	SE	22	31	46	58
	N	9	9	9	9
ENC	$\bar{X}$	381 ³	604	1105	1486
	SE	31	32	52	50
	N	8	8	8	8
EN6	$\bar{X}$	334	546	1143	1476
	SE	38	48	53	84
	N	6	6	8	6
EN12	$\bar{X}$	376 ³	630 ³	1137	1513
	SE	27	40	54	53
	N	9	9	9	9
EXC	$\bar{X}$	320	559	1117	1438
	SE	25	41	45	65
	N	9	9	9	9
EX6	$\bar{X}$	344 ³	575	1088	1433
	SE	22	33	46	61
	N	9	9	9	9
EX12	$\bar{X}$	321	547	1114	1435
	SE	19	36	46	61
	N	9	9	9	9

* First Symbol -

NH₃

C = Normal

EN = ↑ Endogenous

EX = ↑ Exogenous

Second Symbol -

Inhaled H₂SO₄ Concentration

C = 0 mg/m³

6 = 6 mg/m³

12 = 12 mg/m³

Statistical Significance: Superscripts indicate groups which are significantly different ($p < 0.05$; unpaired t test)

1 = CC

3 = C12

5 = EN6

7 = EXC

9 = EX12

2 = C6

4 = ENC

6 = EN12

8 = EX6

Although closing volumes were increased by the same amount following the $10.5 \text{ mg/m}^3 \text{ H}_2\text{SO}_4$ exposures with normal and increased blood ammonia, closing capacity, functional residual capacity, and residual volume were significantly lower following exposures with normal ammonia (C12) compared with increased blood ammonia (EN12). The lower closing capacity indicates that the airways in the lung began to close at lower lung volumes during the C12 than during the EN12 exposures. Since closing volumes in the two groups were the same, the lung was actually drawn to a lower residual volume following the C12 compared with the EN12 exposure (Table 12). Lower values of functional residual capacity following the C12 exposure suggest that the elastic recoil of the lung was increased compared with the EN12 exposure.

Based on these measurements of lung volume, we can predict that inhalation of $10.5 \text{ mg/m}^3 \text{ H}_2\text{SO}_4$ at approximately $2\text{-}\mu\text{m}$ MMAD causes a greater narrowing of small airways than comparable aerosol exposures with blood ammonia increased tenfold. This narrowing is not great enough to produce significant changes in dynamic or quasi-static compliance but it is sufficient to increase the elastic recoil of the lung resulting in decreased values of residual volume and functional residual capacity. The increased closing volumes measured in both $10.5 \text{ mg/m}^3 \text{ H}_2\text{SO}_4$ exposures (C12 and EN12) although of a similar magnitude, do not necessarily indicate similar effects of exposure since the airways actually began closing at lower lung volumes during the C12 exposure.

Because of the different response patterns seen between measurements of closing volume and measurements of other lung volumes no conclusions can be made on the effect of increased blood ammonia on the pulmonary response to inhaled H_2SO_4 aerosols.

Exposure to 5 ppm NH_3 and $15 \text{ mg/m}^3 (\text{NH}_4)_2\text{SO}_4$ produced similar effects on pulmonary function while exposure to $6 \text{ mg/m}^3 (\text{NH}_4)_2\text{SO}_4$ had little effect. During 5 ppm NH_3 and $15 \text{ mg/m}^3 (\text{NH}_4)_2\text{SO}_4$ exposures, the slope of Phase IV of the closing volume maneuver (Table 11, measured as the slope of the volume vs. N_2 curve from closing volume to residual volume) was significantly steeper than following control exposures. This increased slope may reflect an increased gradient in N_2 concentration in the upper (independent) lung regions after airways in the lower (more dependent) regions have closed. Tidal volumes during exposure (Table 6) were also increased in both of these exposure groups compared with control indicating two parameters affected in a similar manner by exposure to 5 ppm NH_3 and $15 \text{ mg/m}^3 (\text{NH}_4)_2\text{SO}_4$.

Similar response to inhaled NH_3 and $(\text{NH}_4)_2\text{SO}_4$ would not be predicted from the expected deposition patterns of these two compounds. Gaseous ammonia is highly soluble and would be expected to be completely absorbed in the nose similar to the absorption of SO_2 measured by Frank et al. (1969). Ammonium sulfate aerosols of $0.4 \mu\text{m}$ MMAD would be expected to

deposit in the deep lung with perhaps only 10 percent deposition in the nose (Task Group on Lung Dynamics, 1966). However, results of these exposures suggest that the fractional deposition of $(\text{NH}_4)_2\text{SO}_4$ in the nose produces irritation similar to that caused by inhalation of 5 ppm NH_3 .

Although responses to inhaled sulfuric acid and ammonium sulfate aerosols were not the same, dogs were affected by 15 mg/m^3 $(\text{NH}_4)_2\text{SO}_4$. Changes caused by ammonium sulfate exposure suggested nasal irritation similar to effects seen during inhalation of 5 ppm NH_3 . Changes caused by H_2SO_4 exposures (e.g., closing volume and lung volume changes) suggested effects on small airways.

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